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Gene for the diphtheria toxin-susceptible elongation factor 2 from *Methanococcus vannielii*

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ABSTRACT

Protein synthesis elongation factor 2 (EF-2) from all archaebacteria so far analysed, is susceptible to inactivation by diphtheria toxin, a property which it shares with EF-2 from the eukaryotic 80S translation system. To resolve the structural basis of diphtheria toxin susceptibility, the structural gene for the EF-2 from an archaebacterium, Methanococcus vannielii, was cloned and its nucleotide sequence determined. It was found that (i) this gene is closely linked to that coding for elongation factor 1α (EF-1α), (ii) the size of the gene product, as derived from the nucleotide sequence, lies between those for EF-2 from eukaryotes and eubacteria, (iii) it displays a higher sequence similarity to eukaryotic EF-2 than to eubacterial homologues, and (iv) the histidine residue which is modified to diphthamide and then ADP-ribosylated by diphtheria toxin is present in a sequence context similar to that of eukaryotic EF-2 but it is not conserved in eubacterial EF-G. The EF-2 gene from Methanococcus is expressed in transformed Saccharomyces cerevisiae but is not ADP-ribosylated by diphtheria toxin. This indicates that the Saccharomyces enzyme system is unable to post-translationally convert the respective histidine residue from the Methanococcus EF-2 into diphthamide.

INTRODUCTION

One of the properties which archaebacteria analysed so far share with eukaryotes is that their protein synthesis elongation factor 2 (EF-2) is inactivated by diphtheria toxin (1). This toxin ADP-ribosylates a diphthamide residue (2) generated from histidine by post-translational modification (3). It has been shown that archaebacterial EF-2 contains such a diphthamide residue (4) and that the amino acid sequence in its immediate vicinity is highly conserved between archaebacteria and eukaryotes (5).

To resolve whether the diphthamide-containing oligopeptide described (5) is located within homologous segments of the

primary structure, whether the gene sequence also codes for a histidine at that position in archaebacteria and which peculiar feature renders the eubacterial homologue (EF-G) insensitive to the action of this toxin, we have cloned and sequenced the gene for EF-2 from Methanococcus (M.) vannielii. The gene was also expressed in Saccharomyces cerevisiae in order to see whether the diphthamide-generating enzyme system from a eukaryotic cell recognizes the archaebacterial target sequence.

MATERIAL AND METHODS

Organisms and plasmids

M. vannielii DSM1224 was grown in the medium described previously (6). E. coli strains JM109 (7) and 490A (8) were used as hosts for transformations. Saccharomyces cerevisiae ATCC 44774 (9) was employed in transformations with plasmid pAAH5 (10). E. coli vectors used were M13mp18, M13mp19 (7), pUC18 and pUC19 (7).

Recombinant DNA techniques

Chromosomal DNA from M. vannielii was isolated as previously described (11). Preparation and purification of plasmid DNA, restriction endonuclease digestions, agarose gel electrophoresis, recovery of DNA fragments from agarose, ligation and transformation experiments were carried out using established procedures (12). Southern hybridization experiments (13) employing homologous DNA were carried out at 68°C in the absence of formamide. Colony hybridization followed the procedure given by Grunstein and Hogness (14).

DNA sequencing

Exonuclease III deletion clones were prepared according to Henikoff (15). They were cloned in both orientations into the multi-linker sites of M13mp18 or pUC19 and sequenced with the chain termination method (16) using the universal or reverse primers (Boehringer Mannheim GmbH). Labelling was with α-[^{35}S] ATP. Alignment and comparison of protein sequences was performed with the aid of a Microvax A computer using the UWGCG programme.

ADP-ribosylation by diphtheria toxin

Saccharomyces cerevisiae ATCC 44774 was transformed (10) with plasmid pAAH5 into which an AccI fragment containing the

EF-2 gene from M. vannielii had been cloned. Leu$^+$ transformants were selected on minimal medium plates.

Transformants were then cultivated in minimal medium to an OD_{420} of 0.5. Cells from 30 ml of the cultures were collected by centrifugation and resuspended in 2 ml Tris/Cl (10 mM, pH 7.5) containing 2 mM 2-mercaptoethanol. Cells from the suspension were broken by two passages through a French press cell at 20,000 p.s.i.. 80 µl of the crude extract were incubated at 37°C for 30 min with 2 µCi [^{32}P] NAD (New England Nuclear, Dreieich) and 1 to 10 µg diphtheria toxin (Calbiochem) in a total volume of 100 µl. The reaction was stopped by the addition of 100 µl double concentrated sample buffer (16). Suitable aliquots were subjected to SDS polyacrylamide gel electrophoresis (7 % acrylamide); the gels were stained with amidoblack, photographed, then dried and used for autoradiography. Extracts from M. vannielii cells were prepared by a single passage through a French press cell at 3,000 p.s.i. and then processed in the same manner as yeast extracts.

RESULTS AND DISCUSSION

Previous work has shown that the gene coding for protein synthesis elongation factor 1α (EF-1α) is localized on a 1.8 kb EcoRI fragment of the M. vannielii chromosome (17). Since, in eubacteria, one of the genes for EF-Tu (the homologue of EF-1α)

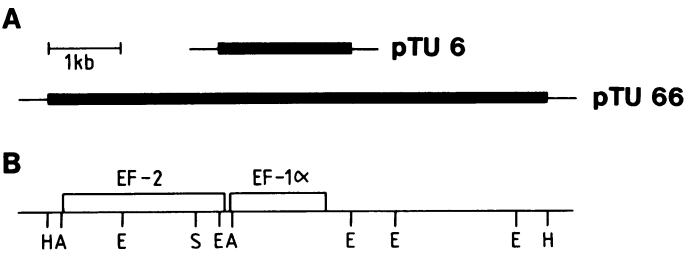

Fig. 1: Cloning of the gene for elongation factor 2 from Methanococcus. A: Methanococcus chromosomal DNA inserts of plasmids pTU6 (17) and pTU66 (this work). B: Physical map of pTU66 showing the restriction sites relevant for this work and the location of the genes for elongation factor 1α and 2. Abbreviations: H: HindIII, A: AccI, S: SacI, E: EcoRI.

is normally linked to that for EF-2 (18), the probability existed that these genes were also linked in Methanococcus.

To test this possibility we used the 1.8 kb EcoRI insert of plasmid pTU6 (ref. 17; Fig. 1) as a hybridization probe in Southern hybridization experiments of chromosomal DNA from Methanococcus digested with various restriction endonucleases. A 6.6 kb HindIII fragment gave a strong signal (17). Chromosomal DNA was subsequently fragmented with endonuclease HindIII and separated by agarose gel electrophoresis and fragments of the appropriate size were recovered from the gel and ligated into pUC18 linearized with HindIII. Clones with the desired insert were obtained by means of colony hybridization using the insert of pTU6 as probe. One of the plasmids obtained (pTU66; Fig. 1a) was used for determining the nucleotide sequence of the DNA segments flanking the gene for EF-1α.

It was found that an open reading frame coding for a protein of molecular weight (80161) preceded the gene for EF-1α with an intergenic region of 132 nucleotides. Its sequence is given in Fig. 2. The size matches that which is predicted for EF-2 of M. vannielii from the results of ADP-ribosylation experiments by diphtheria toxin (1). Unequivocal proof that this open reading frame codes for EF-2 comes from the fact that its sequence contains the tryptic peptide identified by Klink and collaborators as the target of ADP-ribosylation (5) (Fig. 2).

The alignment of the Methanococcus EF-2 sequence with those of EF-2 (EF-G) from eukaryotic and eubacterial origins lends further support to this conclusion. Fig. 2 shows that the overall architecture of the archaebacterial, eubacterial and eukaryotic EF-2 (EF-G) molecules differ considerably, mainly due to the existence of sequence gaps in the archaebacterial and eubacterial proteins. Although regions of sequence similarity are dispersed over the whole length of the primary structure, there is a dense clustering at the N- and C-terminal regions. The N-terminal portion displays sequence motifs which have been shown to be involved in GDP and GTP binding (19); these are the positions 16-22, 87-91 and 141-144 (E. coli nomenclature). The overall sequence similarity of the Methanococcus EF-2 to that of hamster (20) is 35.5 % and to that of E. coli (21) and Micro-

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     Met Gly Arg Arg Ala Lys Met Val Glu Lys Val Lys Ser Leu Met Glu Thr His Asp Gln Ile Arg Asn Met Gly
   1 ATG GGA AGA CGA GCA AAA ATG GTT GAA AAG GTT AAA TCC TTA ATG GAA ACC CAC GAC CAA ATT AGG AAC ATG GGT

     Ile Cys Ala His Ile Ala His Gly Lys Thr Thr Leu Ser Asp Asn Leu Leu Ala Gly Ala Gly Met Ile Ser Lys
  76 ATT TGT GCC CAC ATT GCA CAC GGT AAG ACT ACA TTA TCT GAT AAC TTA CTT GCA GGA GCA GGT ATG ATT TCA AAA

     Asp Leu Ala Gly Asp Gln Leu Ala Leu Asp Phe Asp Glu Glu Glu Ala Ala Arg Gly Ile Thr Ile Tyr Ala Ala
 151 GAT TTA GCA GGA GAC CAA CTT GCA CTT GAC TTC GAT GAA GAA GAA GCT GCA AGA GGT ATT ACA ATC TAT GCT GCA

     Asn Val Ser Met Val His Glu Tyr Asn Gly Lys Glu Tyr Leu Ile Asn Leu Ile Asp Thr Pro Gly His Val Asp
 226 AAC GTT TCA ATG GTA CAC GAA TAT AAT GGA AAA GAG TAT TTA ATT AAC TTA ATT GAT ACA CCA GGT CAC GTT GAC

     Phe Gly Gly Asp Val Thr Arg Ala Met Arg Ala Ile Asp Gly Ala Val Val Val Cys Cys Ala Val Glu Gly Val
 301 TTT GGT GGT GAC GTA ACA AGG GCA ATG AGG GCA ATC GAC GGT GCA GTA GTT GTT TGT TGT GCA GTA GAA GGC GTT

     Met Pro Gln Thr Glu Thr Val Leu Arg Gln Ala Leu Lys Glu Lys Val Lys Pro Val Leu Phe Ile Asn Lys Val
 376 ATG CCT CAG ACT GAA ACT GTT TTA AGA CAG GCG TTA AAA GAA AAA GTT AAA CCT GTT TTG TTT ATT AAC AAA GTT

     Asp Arg Leu Ile Asn Glu Leu Lys Leu Thr Pro Glu Glu Leu Gln Gly Arg Phe Met Lys Ile Ile Ala Glu Val
 451 GAC AGG TTA ATT AAC GAA TTA AAG TTA ACT CCT GAA GAA TTA CAA GGA CGA TTC ATG AAA ATT ATC GCT GAA GTC

     Asn Lys Leu Ile Glu Lys Met Ala Pro Glu Glu Phe Lys Lys Glu Trp Leu Cys Asp Val Val Thr Gly Lys Val
 526 AAT AAA TTA ATC GAA AAA ATG GCT CCA GAA GAA TTT AAG AAA GAA TGG CTC TGT GAT GTA GTC ACT GGA AAA GTT

     Ala Phe Gly Ser Ala Tyr Asn Asn Trp Ala Ile Ser Val Pro Tyr Met Gln Lys Ser Gly Ile Ser Phe Lys Asp
 601 GCT TTT GGT TCA GCA TAC AAT AAC TGG GCA ATT TCA GTT CCA TAC ATG CAG AAA TCA GGA ATT TCA TTT AAA GAC

     Ile Ile Asp Tyr Cys Glu Gln Glu Lys Gln Ser Glu Leu Ala Asp Lys Ala Pro Leu His Glu Val Ile Leu Asp
 676 ATT ATT GAC TAC TGT GAA CAG GAA AAA CAG TCA GAA TTA GCT GAT AAA GCT CCA CTT CAC GAA GTT ATT CTT GAC

     Met Ala Ile Lys His Leu Pro Asn Pro Leu Gln Ala Gln Lys Tyr Arg Ile Pro Asn Ile Trp Lys Gly Asp Ala
 751 ATG GCA ATC AAA CAC TTG CCA AAC CCA CTC CAA GCT CAA AAA TAC AGA ATT CCA AAC ATT TGG AAG GGA GAT GCA

     Glu Ser Glu Val Gly Lys Ser Met Ala Met Cys Asp Pro Asn Gly Pro Leu Ala Gly Val Val Thr Lys Ile Ile
 826 GAA TCT GAA GTT GGT AAA TCC ATG GCA ATG TGT GAC CCT AAT GGA CCA CTT GCA GGT GTT GTT ACA AAA ATT ATT

     Val Asp Lys His Ala Gly Ser Ile Ser Ala Cys Arg Leu Phe Ser Gly Arg Ile Lys Gln Gly Asp Glu Leu Tyr
 901 GTT GAC AAA CAC GCA GGT TCA ATT TCA GCA TGC AGA TTG TTT TCT GGA AGA ATC AAA CAA GGT GAC GAA TTA TAC

     Leu Val Gly Ser Lys Gln Lys Ala Arg Ala Gln Gln Val Ala Ile Phe Met Gly Ala Glu Arg Val Gln Val Pro
 976 CTT GTA GGC TCT AAA CAA AAG GCA AGA GCA CAA CAA GTT GCT ATC TTC ATG GGT GCT GAA AGA GTT CAA GTG CCA

     Ser Ile Ser Ala Gly Asn Ile Cys Ala Leu Thr Gly Leu Arg Glu Ala Thr Ala Gly Glu Thr Val Cys Ser Pro
1051 AGC ATT TCC GCA GGA AAC ATT TGT GCA TTA ACG GGT TTA AGA GAA GCT ACT GCT GGA GAA ACC GTA TGT AGC CCA

     Ser Lys Ile Leu Glu Pro Gly Phe Glu Ser Leu Thr His Thr Ser Glu Pro Val Ile Thr Val Ala Ile Glu Ala
1126 TCA AAA ATT TTA GAA CCC GGA TTT GAA TCT TTG ACC CAC ACA TCT GAA CCA GTA ATT ACT GTT GCA ATC GAA GCT

     Lys Asn Thr Lys Asp Leu Pro Lys Leu Ile Glu Ile Leu Arg Gln Ile Gly Arg Glu Asp Asn Thr Val Arg Ile
1201 AAA AAC ACG AAA GAT TTA CCG AAA TTA ATC GAG ATT TTA AGC CAG ATT GGA AGA GAA GAT AAC ACT GTA AGA ATC

     Glu Ile Asn Glu Glu Thr Gly Glu His Leu Ile Ser Gly Met Gly Glu Leu His Ile Glu Val Ile Thr Asp Thr
1276 GAA ATC AAT GAA GAA ACC GGT GAA CAC TTA ATC AGC GGT ATG GGT GAA CTC CAC ATT GAA GTT ATC ACA GAT ACC

     Lys Ile Gly Arg Asp Gly Gly Ile Glu Val Asp Val Gly Glu Pro Ile Ile Val Tyr Arg Glu Thr Ile Thr Gly
1350 AAG ATT GGA AGG GAC GGT GGA ATT GAA GTA GAT GTT GGT GAA CCA ATT ATC GTT TAT AGG GAA ACC ATT ACA GGA

     Thr Ser Pro Glu Ile Glu Gly Lys Ser Pro Asn Lys His Asn Lys Leu Tyr Met Ile Ala Glu Pro Met Glu Glu
1426 ACT TCC CCT GAA ATT GAA GGA AAA TCA CCA AAC AAG CAC AAC AAG CTC TAC ATG ATT GCA GAA CCA ATG GAA GAG

     Ser Val Tyr Ala Ala Tyr Val Glu Gly Lys Ile His Asp Glu Asp Phe Lys Lys Lys Thr Asn Val Asp Ala Glu
1501 TCA GTT TAT GCA GCA TAC GTT GAA GGT AAA ATC CAC GAT GAA GAC TTC AAA AAG AAG ACT AAC GTA GAT GCA GAG

     Thr Arg Leu Ile Glu Ala Gly Leu Glu Arg Glu Gln Ala Lys Lys Val Met Ser Ile Tyr Asn Gly Asn Met Ile
1576 ACT AGG TTA ATC GAA GCT GGA CTT GAA AGA GAA CAG GCT AAA AAA GTT ATG TCA ATT TAC AAT GGA AAC ATG ATT

     Val Asn Met Thr Lys Gly Ile Val Gln Leu Asp Glu Ala Arg Glu Leu Ile Glu Gly Phe Lys Glu Gly Val
1651 GTA AAC ATG ACA AAA GGT ATT GTT CAA CTC GAT GAA GCA AGA GAA TTA ATC ATT GAG GGT TTC AAA GAA GGT GTA

     Lys Gly Gly Pro Leu Ala Ser Glu Arg Ala Gln Gly Val Lys Ile Lys Leu Ile Asp Ala Thr Phe His Glu Asp
1726 AAA GGA GGG CCA CTT GCA TCT GAA AGA GCT CAA GGA GTA AAA ATC AAA CTA ATT GAT GCA ACT TTC CAC GAA GAT

     Ala Ile His Arg Gly Pro Ser Gln Ile Ile Pro Ala Ile Arg Phe Gly Val Arg Asp Ala Val Ser Ser Ala Lys
1801 GCA ATC CAC AGA GGG CCT TCA CAA ATC ATC CCT GCA ATT AGA TTT GGA GTA AGA GAT GCA GTT TCA AGT GCA AAA

     Pro Ile Leu Leu Glu Pro Met Gln Lys Ile Tyr Ile Asn Thr Pro Gln Asp Tyr Met Gly Asp Ala Ile Arg Glu
1876 CCA ATT CTC TTG GAA CCT ATG CAA AAA ATT TAC ATT AAC ACG CCA CAG GAT TAC ATG GGT GAT GCG ATC AGA GAA

     Ile Asn Asn Arg Arg Gly Gln Ile Val Asp Met Glu Gln Glu Gly Asp Met Ala Ile Ile Lys Gly Ser Val Pro
1951 ATC AAC AAC AGG AGA GGG CAG ATT GTT GAC ATG GAA CAG GAA GGT GAC ATG GCA ATT ATC AAA GGA AGT GTT CCT

     Val Ala Glu Met Phe Gly Phe Ala Gly Ala Ile Arg Gly Ala Thr Gln Gly Arg Cys Leu Trp Ser Val Glu Phe
2026 GTT GCT GAA ATG TTT GGA TTC GCT GGT GCA ATT CGT GGT GCA ACC CAA GGT AGA TGT TTA TGG AGT GTT GAA TTC

     Ser Gly Phe Glu Arg Val Pro Asn Glu Ile Gln Thr Lys Val Val Ala Gln Ile Arg Asp Arg Lys Gly Leu Lys
2101 TCA GGA TTC GAA AGA GTA CCA AAT GAA ATT CAA ACT AAG GTT GTT GCT CAA ATT AGA GAC AGA AAA GGT TTA AAA

     Ser Glu ###
2176 TCA GAA TAA
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Fig. 2: Nucleotide sequence and derived amino acid sequence of the EF-2 gene. The tryptic peptide identified by Gehrmann et al. (5) as the site of ADP-ribosylation by diphtheria toxin is boxed; the histidine residue converted to diphthamide is circled.

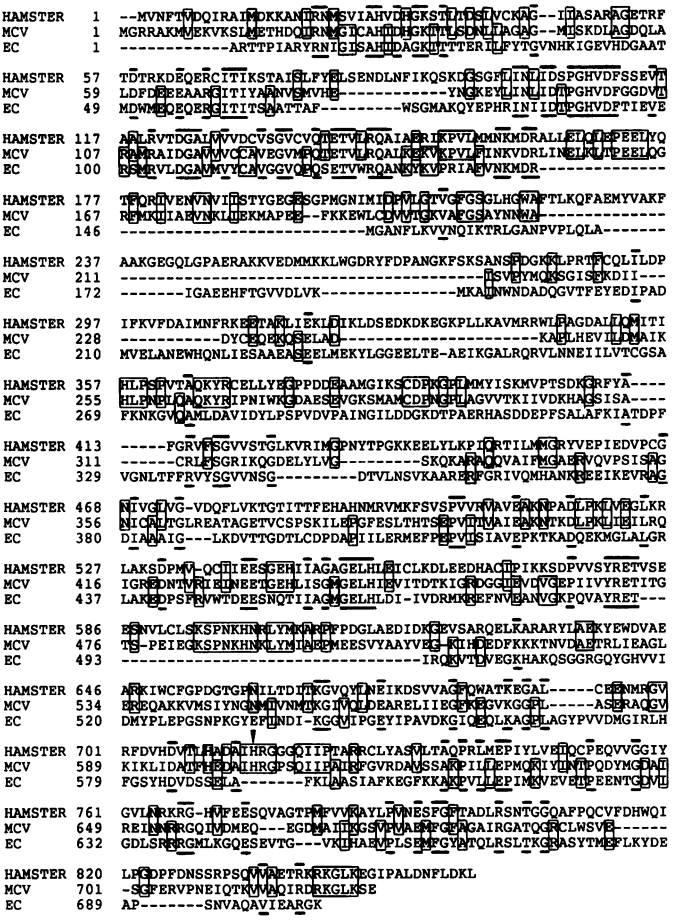

Fig. 3: Alignment (Programme ALIGN) of the Methanococcus (MCV) EF-2 sequence with that of EF-2 from hamster (20) and of EF-G from E. coli (21). Amino acid positions conserved in all three sequences are indicated by lines above and below the sequences. Positions shared by the Methanococcus and hamster sequences on one side or by those from Methanococcus and E. coli are boxed. The position of the histidine converted into diphthamide is marked by an arrow.

coccus (22) (alignment not shown) 32.2 % and 32.8 %, respectively. EF-2 from Methanococcus, therefore, differs significantly in its phylogenetic distance to the homologues of the other two lineages when compared with the phylogenetic behaviour displayed by EF-1α (17).

The tryptic peptide, determined by Gehrmann et al. (5) as

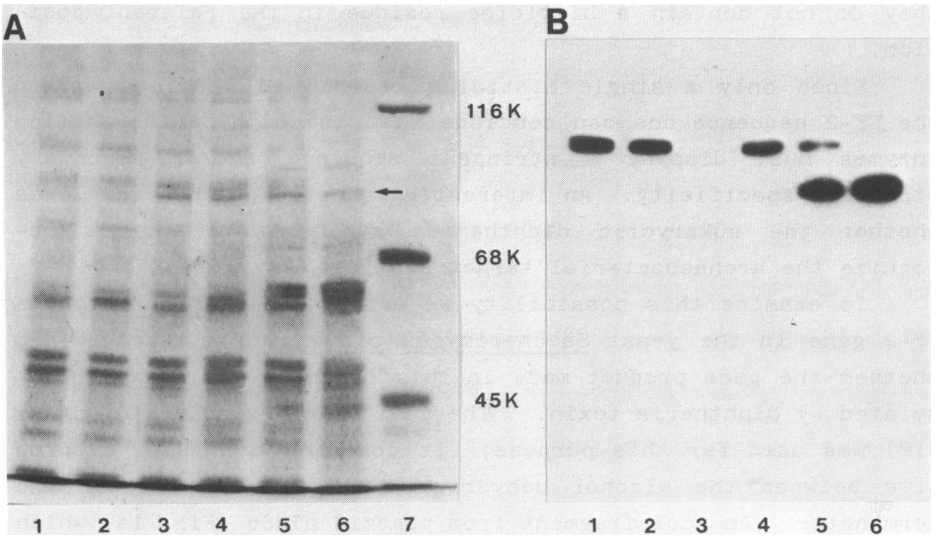

Fig. 4: ADP-ribosylation by diphtheria toxin. Cell extracts from S. cerevisiae ATCC 44774 or Methanococcus vannielii were incubated with diphtheria toxin and [^{32}P]NAD and separated in 7 % polyacrylamide gels in the presence of SDS. Part A: Amidoblack stained gel. The arrow indicates the migration position of the presumptive EF-2 gene product. Part B: Autoradiogram of the gel after drying. Lanes contain the following extracts: (1) S. cerevisiae harboring plasmid pAAH5 without insert, (2) like lane 1 with pAAH5 containing the Methanococcus EF-2 gene in the inverse orientation; (3 and 4) like lane 2 but with the EF-2 gene in the correct orientation, (5) a 1:1 mixture of extracts from Methanococcus and S. cerevisiae containing pAAH5 with the correctly oriented EF-2 gene, (6) Methanococcus. Lane 7 gives a molecular weight standard. Extract separated in lane 3 was incubated with [^{32}P]NAD in the absence of diphtheria toxin.

the target of ADP-ribosylation by diphtheria toxin, comprises the amino acids from position 592 to 604 (Fig. 3). There is a histidine residue (His-603) in the position where diphthamide was identified in the amino acid sequence (Fig. 3). As in eukaryotic EF-2 sequences, histidine is, therefore, post-translationally modified to diphthamide. A detailed comparison of this particular segment of EF-2 within all the EF-2 sequences determined so far (20-22) shows that there is considerable sequence similarity - but not complete identity - in the archaebacterial and eukaryotic molecules. The two eubacterial versions lack a homologous sequence stretch and, significantly,

they do not contain a histidine residue in the relevant position.

Since only a single histidine residue is modified within the EF-2 sequence one can conclude that the diphthamide-forming enzymes must display a stringent sequence and/or secondary structure specificity. An interesting question, therefore, was whether the eukaryotic diphthamide synthesizing enzymes recognize the archaebacterial target peptide.

To examine this possibility we expressed the Methanococcus EF-2 gene in the yeast Saccharomyces cerevisiae and determined whether the gene product made in this organism can be ADP-ribosylated by diphtheria toxin. The yeast expression vector pAAH5 (10) was used for this purpose; it contains a HindIII cloning site between the alcohol dehydrogenase I (ADHI) promoter and terminator. An AccI fragment from plasmid pTU66 (Fig. 1a) which contains the complete EF-2 gene from Methanococcus plus 42 bp of the 5' flanking region was isolated and ligated into HindIII-linearized vector pAAH5 by blunt-end ligation. The ligation mixture was used to transform E. coli 490A. Clones were isolated which contained the AccI fragment in the correct or the inverse orientation relative to the alcohol dehydrogenase gene promoter.

Plasmids containing the correctly or inversely oriented AccI inserts were then transformed into Saccharomyces cerevisiae ATCC 44774. Crude extracts from each were analysed for ADP-ribosylation by diphtheria toxin. Extracts from S. cerevisiae containing the vector alone or from M. vannielii were used as controls.

Fig. 4 gives the results. They show that diphtheria toxin ADP-ribosylates proteins in the control extracts which correspond in their size to those of the expected EF-2 gene products from S. cerevisiae (lanes 1, 2) and Methanococcus (lane 6). Yeast transformants carrying pAAH5 with the Methanococcus DNA insert in the correct orientation relative to the ADHI promoter express a protein co-migrating in SDS gels with EF-2 from Methanococcus (see lanes 3-6). This protein formed in yeast, however, is not modified by diphtheria toxin (lane 4). A plausible conclusion, therefore, is that it lacks the diphthamide residue.

Evidence that the protein expressed from the correctly oriented insert is indeed EF-2 from Methanococcus comes - apart from its size - from the fact that it is not formed by cells containing vector pAAH5 (lane 1) or the plasmid with the oppositely oriented AccI fragment (lane 2).

Control experiments (not shown) were also performed to analyse whether the lack of ADP-ribosylation of Methanococcus EF-2 formed in yeast is due to aggregation or precipitation of the heterologous gene product. Electrophoretic analysis of 100,000 x g supernatant from the transformants demonstrated, however, that this protein is present in the supernatant.

At present, it is not known whether the diphthamide-forming enzyme system recognizes primary and/or secondary structure features of the target protein. Assuming, therefore, that the heterologous gene product is correctly folded, one can conclude that the diphthamide-forming system of yeast does not recognize the Methanococcus target sequence. If this is correct then this suggests that ADP-ribosylation by diphtheria toxin depends less stringently on the EF-2 sequence context than on formation of the diphthamide.

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REFERENCES

1. Klink, F. (1985) in The Bacteria VIII. Archaebacteria, Woese, C.R. and Wolfe, R.S. (eds), Academic Press, New York, London, pp. 379-410.
2. Brown, P.A. and Bodley, J.W. (1979) FEBS Lett. 103, 253-255.
3. Van Ness, B.G., Howard, J.B. and Bodley, J.W. (1980) J. Biol. Chem. 255, 10710-10716.
4. Pappenheimer, A.M., Dunlop, P.C., Adolph, K.W. and Bodley, J.W. (1983) J. Bact. 153, 1342-1347.

5. Gehrmann, R., Henschen, A. and Klink, F. (1985) FEBS Lett. 185, 37-42.
6. Douglas, C., Achatz, F. and Böck, A. (1980) Zbl. Bakt. Hyg. I. Abt. Orig. C1, 1-11.
7. Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 33, 103-109.
8. Landsmann, J., Kröger, M. and Hobom, G. (1982) Gene 20, 11-24.
9. Botstein, D. (1979) Gene 8, 17-24.
10. Ammerer, G. (1983) Methods Enzymol. 101, 192-201.
11. Jarsch, M., Altenbuchner, J. and Böck, A. (1983) Mol. Gen. Genet. 189, 41-47.
12. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in Molecular Cloning, Cold Spring Harbor Laboratories, New York.
13. Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
14. Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965.
15. Henikoff, S. (1984) Gene 28, 351-359.
16. Laemmli, U.K. (1970) Nature 227, 680-685.
17. Lechner K. and Böck, A. (1987) Mol. Gen. Genet. 208, 523-528.
18. Nomura, M., Gourse, R. and Baughman, G. (1984) Ann. Rev. Biochem. 53, 75-117.
19. Dever, T.E., Glynias, M.J. and Merrick, W.C. (1987) Proc. Natl. Acad. Sci. USA 84, 1814-1818.
20. Khono, K., Uchida, T., Ohkubo, H., Nakanishi, S., Fukui,T., Ohtsuka, E., Ikehara, M. and Okada, Y. (1986) Proc. Natl. Acad. Sci. USA 83, 4978-4982.
21. Zengel, J.M., Archer, R.H. and Lindahl, L. (1984) Nucl. Acids Res. 12, 2181-2192.
22. Ohama, T., Yamav, F., Muto, A. and Osawa, S. (1987) J. Bact. 169, 4770-4777.